

NITROGEN and OXYGEN ISOTOPE METHODS

Nitrate, ammonia and phosphate in surface and groundwater

Quality Assurance Plan

UNL WATER SCIENCES LABORATORY

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Sampling

Water samples for $\delta^{15}\text{N}$ of nitrate and ammonia are collected and stored in 1-liter polyethylene bottles. Samples specifically collected for $\delta^{15}\text{N}$ analysis of ammonia-N in ground-water may be acidified to pH <2.0 with concentrated sulfuric acid to prevent volatilization, samples for $\delta^{15}\text{N}$ analysis of nitrate-N can be collected without acidification. Water samples for $\delta^{18}\text{O}$ analysis of nitrate or phosphate must be collected and frozen with no acid preservation. Freezing is typically used as an alternative to field filtering and acidification for all three methods.

All samples should be stored and transported on ice. Samples must be frozen before transport if holding times beyond 24 hours are anticipated. As with other types of laboratory analyses, the objectives are that measurements be representative of the sample at the time of analysis, and that the results be comparable to those obtained by other laboratories when identical samples are analyzed. A representative sample is dependent upon proper collection, labeling, transport, storage, and analysis. The comparability of results is fostered through the use of accepted sampling and analytical protocols. The Water Sciences Laboratory cannot be responsible for the validity of sampling procedures when samples are not collected by WSL personnel.

All samples transported to the Water Sciences Laboratory will be brought to the attention of the laboratory director and the isotope chemist. Samples should be handled so that their integrity is assured. Personnel accepting samples and field records at the Water Sciences Laboratory are responsible for:

1. initiating the laboratory record of the samples and insuring that the handling of samples is documented;
2. logging sample information on tracking forms, and assigning a LIMS sample ID;
3. storing samples in the proper location;
4. alerting the analyst to the presence and status of the samples.

Laboratory Quality Assurance

The goal of the quality assurance plan is to ensure that all environmental data are scientifically valid, defensible, and of known precision and accuracy. This goal is achieved by ensuring that adequate quality assurance steps and procedures are used throughout the entire analytical process. Under the supervision of the laboratory services director, laboratory personnel

1. receive and log-in samples for testing as specified in the standard operating procedure for sample receipt. Those samples not received under satisfactory conditions for testing are rejected according to established protocol. Samples of poor quality, as described in the criteria for rejection of samples in the approved laboratory protocol, will be accepted in the laboratory but may not be accepted for analysis and repeat sampling will be requested.

If a sample is not acceptable, it will not be automatically discarded by the laboratory. Samples are scheduled for testing within accepted holding times.

2. perform analyses according to standard operating procedures (SOPs);
3. maintain instruments, perform quality control program tasks, and other laboratory tasks according to laboratory position specifications;
4. report laboratory results according to protocol. Test results are the property of the test requester, not the laboratory. The laboratory is the custodian of the results and must be responsible to the test requester for their handling. Test results are released only to the test requester. Release of results in any other manner (including subpoenas) must be arranged through the laboratory director. Questionable results (those significantly outside of the expected range or those where quality control indicates another test is necessary) will be held and, if appropriate, the sample will be retested.

Analytical Procedures

Nitrogen-15 analysis of nitrate and ammonia. Sample preparation for nitrogen isotope analysis involves steam distillation, quantitative conversion to nitrogen gas which is purified and collected on a high vacuum preparation system, and measurement by isotope ratio mass spectrometry. Frozen samples are thawed immediately before analysis and shaken thoroughly to insure homogeneity prior to measurement of an aliquot into a distillation flask. In samples for determination of $\delta^{15}\text{N}$ of nitrate-N, the pH of the solution is increased to 9.5-10 with the addition of magnesium oxide and ~50 mL of ammonia distillate are collected and discarded. Nitrate in the solution is then reduced to ammonia with Devardas alloy, 100 mL of distillate collected in a boric acid indicator solution, and the nitrate-N concentration determined titrimetrically with standardized sulfuric acid. In samples for $\delta^{15}\text{N}$ determination of ammonia-N, an aliquot of the acidified sample is neutralized with a known amount of 1N sodium hydroxide. A second 100-mL aliquot is put on the distillation system, the predetermined amount of sodium hydroxide added to neutralize the sample, the pH increased to 9.5-10 with magnesium oxide, and 100 mL of ammonia distillate collected in a boric acid indicator solution. The ammonia-N concentration is determined titrimetrically with standardized sulfuric acid.

A nitrate-N standard, a blank, and an ammonium-N standard of known isotopic composition and concentration are distilled prior to beginning the day's analyses. After every fifth sample, the system is cleaned by steam distilling ethanol. One sample in twenty (5%) is analyzed in replicate to monitor precision. The distillates from both the ammonia and nitrate fractions are acidified with 1N sulfuric acid immediately after titration and evaporated to 1 to 2 mL on a hot plate. The concentrated distillate is reacted with lithium hypobromite on a high-vacuum preparation line and the ammonium quantitatively reduced to nitrogen gas, purified by passage through two liquid nitrogen cryotrap and a 400°C copper oven, and collected in a gas sample bulb. Atmospheric nitrogen standards are prepared on the same high-vacuum preparation line. With the exception of an additional copper oxide furnace trap, gas purification is similar to that of the samples. Ultrapure tank nitrogen is used as the working standard and is calibrated against the atmospheric nitrogen standard.

Nitrogen Isotope Instrumentation. All nitrogen isotope measurements are performed on either a VG Instruments OPTIMA or a GVI Isoprime dual inlet stable isotope ratio mass spectrometer

(IRMS). The OPTIMA was purchased in 1992 as a state-of-the-art IRMS with improved ion optics, amplifier design, and electronics which improved both the sensitivity and precision of nitrogen isotope ratio measurements. The instrument is capable of measurements in the mass range of 2-80 m/z, with a resolution (M/ΔM) of 100. It has an absolute sensitivity of 1500 molecules of CO₂ per mass 44 ion. It has been fitted with a nitrogen “cold finger” cryo-trap which reduces the sample size requirements for nitrogen to 20 bar μL, which is equivalent to ~23 μg N, or about 0.2 mg/L NO₃-N in an unconcentrated sample. The internal precision of the OPTIMA is 0.01 ‰ (2σ₁₀). The GVI Isoprime was purchased in 2007 with specifications that match or exceed the OPTIMA.

The isotopic composition of the purified nitrogen samples are measured and simultaneously compared to that of the working standard. The δ¹⁵N of the sample is the measured isotopic composition relative to the atmospheric standard expressed in parts per thousand (‰) using the equation:

$$\delta^{15}\text{N} (\text{‰}) = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \times 1000$$

The sample standard deviation for 20 preparations of an ammonium standard of known isotopic composition is ±0.5‰. The standard deviation of a similar number of nitrate-N working standards is ±0.6‰. The standard deviation for 216 preparations of the working gas standard was ±0.05‰. The relative percent difference from the analysis of 39 sets of duplicate samples was 0.59‰.

Interlaboratory Comparison. In 1993, the Water Sciences Laboratory participated in an interlaboratory comparison between 15 laboratories world-wide in the measurement of seven different reference materials for nitrogen isotope ratio analysis (Böhlke and Coplen, 1995). The following table lists the reported interlaboratory averages compared with the results submitted by the Water Sciences Laboratory:

Identification	Substance	δ ¹⁵ N (‰)	δ ¹⁵ N (‰)
		Interlaboratory Average	Water Sciences Laboratory
NSVEC	N ₂ Gas	-2.77 ± 0.05	-2.70 ± 0.06
IAEA-N1	NH ₄ (SO ₄)	+0.43 ± 0.07	+0.38 ± 0.14
IAEA-N2	NH ₄ (SO ₄)	+20.32 ± 0.09	+20.14 ± 0.06
IAEA-N3	KNO ₃	+4.69 ± 0.09	+4.14 ± 0.12
USGS25	NH ₄ (SO ₄)	-30.25 ± 0.38	-30.16 ± 0.09
USGS26	NH ₄ (SO ₄)	+53.62 ± 0.25	+53.21 ± 0.15
USGS32	KNO ₃	+179.2 ± 1.3	+177.02 ± 0.24

These reference standards continue to be used as verification of the accuracy of the nitrogen isotope analysis at the Water Sciences Laboratory.

Oxygen-18 analysis of nitrate. Samples for determination of oxygen isotope composition of nitrate are prepared according to Chang et al 1999 and Silva et al 2000. Briefly, a measured volume of sample containing 0.25 mg NO₃-N is treated with 1M barium chloride to precipitate sulfate and phosphate. The solution is then filtered, and then passed through a cation exchange column to remove excess Ba²⁺, and then through an anion exchange column to concentrate

nitrate. Nitrate is then eluted using 3 M hydrochloric acid, neutralized with Ag₂O, filtered to remove the AgCl precipitate, and then dried to producing purified AgNO₃. The AgNO₃ is then dissolved in 1ml of reagent water and 100 µL (25 µgN) aliquots transferred to three silver cups and dried for analysis by pyrolysis on nickelized a Eurovector E. The AgNO₃ can then be analyzed for oxygen isotope composition using high temperature pyrolysis on nickelized graphite in a closed tube to produce CO on a Eurovector EA Isoprime continuous flow isotope ratio mass spectrometer. The final result is averaged from the triple instrumental results and converted to the standard oxygen isotope reference (VSMOW =0.00 ‰).

$$\delta^{18}\text{O} (\text{‰}) = \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}} - (^{18}\text{O}/^{16}\text{O})_{\text{standard}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} \times 1000$$

A reagent grade potassium nitrate (KNO₃) is used as a working standard, and reference sucrose oxygen isotope standard (obtained from Elemental Microanalysis) are analyzed with every sample batch (up to 20 samples) both to calibration and for drift correction. USGS 34 and USGS 35 reference standards are analyzed at least monthly to compare and convert working standards to a δ¹⁸O isotope value with respect to VSMOW. The 1σ measured analytical precision of δ¹⁸O is ±0.5‰ for solutions of KNO₃ standard processed through the entire procedure. In addition to triplicate instrumental average measurement, laboratory duplicates are carried through the preparation process and analyzed at a rate of 5%.

Oxygen-18 Analysis of Phosphate. Soluble phosphate (PO₄-P) in water or extracts is coprecipitated with Mg(OH)₂ according to the preparation methods described in McLaughlin et al (2004) and McLaughlin et al (2006). The flock is allowed to settle overnight, separated by centrifuging and then dissolved using a mixture of acetic and nitric acid. The solution is then buffered to pH=5.5 with 5M potassium acetate, and then mixed with cerium nitrate resulting in precipitation of cerium phosphate. The CePO₄ separated by centrifugation, rinsed with 1M potassium acetate to remove chloride, and then dissolved in nitric acid. Cerium ions are separated using cation exchange resin and final purified eluent neutralized. Silver nitrate is added to the sample to precipitate solid silver phosphate. The AgPO₄ is centrifuged and rinsed and 1 ml of 15% H₂O₂ is added to oxidize remaining natural organic matter. The AgPO₄ is then vacuum filtered onto 0.45 µm silver filters. The silver phosphate is then weighed to determine yield and subsequently analyzed for oxygen isotope composition using high temperature pyrolysis on nickelized graphite in a closed tube to produce CO on a Eurovector EA Isoprime continuous flow isotope ratio mass spectrometer. A working potassium phosphate (KH₂PO₄) and sucrose reference standard (obtained from Elemental Microanalysis) are analyzed with each sample batch of up to 20 samples. USGS 34 and USGS 35 are used to convert δ¹⁸O isotope results of working standard to the reference for oxygen isotope results as describe for nitrate. Replicate analysis of the working standard indicates the precision to be ±0.6‰. Laboratory duplicates are carried through the preparation process and analyzed at a rate of 5%.

Overall Laboratory Quality Control Procedures

The WSL quality control checks for samples include, but are not limited to:

1. the use of laboratory forms to identify date (month/day/year) and time of sample receipt,

- the recipient, the date of analysis and the analyst, and the test result;
- 2. strict adherence to chain of custody procedures (when required);
- 3. presenting sampling instructions and training sessions to field personnel (when required);
- 4. inserting known blind check samples to accompany samples to verify proper sample transit conditions, if required by the approved laboratory protocol.

Analytical Quality Control Checks

The laboratory's analytical quality control checks include, but are not limited to:

- 1. the analyst's strict adherence to approved or accepted laboratory protocols;
- 2. analysis of method or reagent blanks (5%) with each run;
- 3. analysis of field blanks (5%), sample blanks, and equipment check blanks;
- 4. analysis of field duplicates (5%) or replicate samples (5%);
- 5. analysis of certified QC standards, if available, to verify accuracy;
- 6. proper instrument calibrations and standardization as specified in the instrument manual and the approved laboratory protocol using known standards for preparing calibration curves to calculate sample results;
- 7. plotting quality control results prior to calculating sample results to verify the results are within established ranges;
- 8. flagging all results not within expected ranges for the particular analyte;
- 9. secondary review of all laboratory results prior to reporting.

Assessment of Quality Control

The acceptability of duplicate, replicate, and reference sample results are evaluated by the use of Shewhart Statistical Equations and quality control charts. Reference for this section can be made to: "The Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, Control of Analytical Performance, by the Analytical Quality Control Laboratory, (EPA-600/4-79-019).

Precision of the analytical measurements from duplicate analyses are assessed using the following Shewhart Equations:

- 1. Determine I for duplicates

$$I = \frac{|A - B|}{(A + B)}$$

where A and B are the analytical results from the two duplicate samples;

- 2. Determine range, R of duplicates

$$R = |A - B|$$

where A and B are the analytical results from the two duplicates. Plot R as one value per duplicate

Corrective Action

Laboratory corrective action will be initiated by the laboratory director under the following circumstances:

1. unacceptable precision between duplicate analyses (values exceeding control limits);
2. failure to pass external testing audits;
3. use of nonstandard procedures, i.e., not following the approved laboratory protocol.

If corrective action is necessary, the analyst will take some or all of the following steps to remedy the problem:

1. recheck all calculations and measurement data;
2. check instruments to ensure proper calibration and operation;
3. check reagents for proper reactivity;
4. assure that proper operating procedures are followed;
5. reanalyze all samples run at the same time the problem occurred;
6. if the problem continues, seek assistance from instrument manufacturer as necessary.

Preventative Maintenance

Preventive maintenance will be performed by the isotope chemist or laboratory services director as specified in the instrument service manual. The objective of preventive maintenance is to keep the instrument in peak operational condition. All preventive maintenance for the instrument will be documented in a permanent record routinely evaluated by the laboratory quality assurance coordinator. If the instrument requires non-routine maintenance, it will be repaired as per the manufacturer or vendor service contract or returned to the manufacturer for service and repair.

Communication/Reporting

The isotope chemist will advise the laboratory director and other responsible program management of the performance of the data production systems and of any laboratory analytical problems. All sample information and results are managed through the use of data management software which includes a in-house developed Laboratory Information Management System (LIMS) to facilitate data sorting and preparation of reports. Precautions are taken during the reduction, manipulation, and storage of data to prevent the introduction of errors or the loss or misinterpretation of the data. The lab services director will conduct periodic inspection of the data flow process to ensure accurate intralaboratory communication.

References

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